510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k031308

B. Analyte:
Anti-mitochondrial antibody

C. Type of Test:
Semi-quantitative ELISA

D. Applicant:
RhiGene, Inc.

E. Proprietary and Established Names:
MESACUP-2 Test Mitochondria M2

F. Regulatory Information:

1. Regulation section:
   21 CFR §866.5090 Anti-mitochondrial Antibody Immunological Test System
2. Classification:
   Class II
3. Product Code:
   DBM
4. Panel:
   IM 82

G. Intended Use:

1. Intended use(s):
   The MESACUP-2 Test Mitochondria M2 is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of anti-Mitochondrial antibodies in human serum as an aid in the diagnosis of primary biliary cirrhosis.
2. Indication(s) for use:
   The MESACUP-2 Test Mitochondria M2 is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of anti-Mitochondrial antibodies in human serum as an aid in the diagnosis of primary biliary cirrhosis.
   The MESACUP-2 Test Mitochondria M2 is intended to be used by clinical (hospital and reference) laboratories.
3. Special condition for use statement(s):
   The device is for prescription use only.
4. Special instrument Requirements:
   None

H. Device Description:

The device is an enzyme-linked immunosorbent assay (ELISA) using microtiter plates as the solid phase. The plate wells are coated with recombinant M2 antigen

which captures mitochondrial (M2) autoantibodies present in the patient sample. The conjugate is polyclonal goat anti-human IgG, IgM and IgA (heavy chain specific) horseradish peroxidase (HRP) which uses 3,3'5,5' tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/H_2O_2) as substrate. The kit contains 2 levels of calibrators (0 units/mL and 100 u/mL) for interpretation of results. A positive and a negative control are included with the kit. The kit also contains sample diluent, wash buffer concentrate and stop solution.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Quanta Lite Mitochondria M2 ELISA from INOVA Diagnostics
2. Predicate K number(s):
   K933180
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Indications for Use	For detection of anti-mitochondrial antibodies as an aid in the diagnosis of primary biliary cirrhosis	Same
Assay principle	Indirect ELISA	Same
Sample matrix	Serum	Same
Substrate	TMB	Same
Differences		
Item	**Device**	**Predicate**
Cut-off	7 U/mL	1.0 Units
Detection range	0-300 U/mL	0-6 Units
Assay time	150 minutes	90 minutes
Conjugate	HRP-goat anti-human IgG/IgM/IgA	HRP-goat anti-human IgG

J. Standard/Guidance Document Referenced (if applicable):
Not applicable

K. Test Principle:
Enzyme-linked immunosorbent assay (ELISA) technology is a well established methodology.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*
   Three lots of the MESACUP-2 Test Mitochondria M2 were performed to determine the assay's intra-assay, inter-assay and inter-lot value precision.

Intra-assay

Intra-assay precision (%CV) was determined by running 3 serum samples (low, moderate and high positive) using 8 dilutions of 3 different coated plates from 3 different plate lots. The 3 separate plates employed were randomly selected from each plate-coating run (kit-lot). Resulting %CVs were determined from values obtained. The mean intra-assay precision for the 3 samples tested on 3 plates from each lot was 1.9% ranging from 0.9-3.4%.

Inter-assay, intra-lot

To determine the amount of variability between plates of the same lot, 3 samples in duplicate were tested on 6 separate assays employing 6 different plates randomly selected from the same plate lot. This was performed on 3 separate plate lots. The mean %CV for inter-assay, intra-lot precision was 2.5% with a range of 1.0-4.3%.

Inter-assay, inter-lot

The precision between lots was determined by comparing the values recovered for 3 different samples on 3 different pilot lots. Each of the 3 samples was tested in duplicate and by 2 operators in each assay. The mean inter-assay, inter-lot %CV was 5.3%.

b. *Linearity/assay reportable range:*
The reportable range of 5-300 U/mL was demonstrated by recovery studies.

c. *Traceability (controls, calibrators, or method):*
An international reference material for anti-mitochondrial M2 antibodies is not available. The assay is calibrated in relative arbitrary units.

d. *Detection limit:*
Not applicable

e. *Analytical specificity:*
Hemoglobin (up to 480 mg/dL), bilirubin (up to 20 mg/dL), chyle (up to 2780 units as Formazine) and Rheumatoid Factor (up to 520 IU/mL) do not interfere with the assay.

f. *Assay cut-off:*
A healthy population consisting of 168 unselected human serum samples was tested for anti-mitochondria M2 antibodies. All normal samples were tested in duplicate. The cut-off was established by calculating the number and percentage of positive samples in the challenge population at the mean plus 3 standard deviation (SD) intervals. The cut-off value of 7 U/mL was assigned to the mean plus 3 SD interval OD and value. Using 7 U/mL as the cut-off, the assay was 98.2% specific. A second set of 40 healthy blood donor samples was tested for anti-mitochondria M2 antibodies. All normal

samples were tested in duplicate. The number and percent positive samples confirmed value recovery of a normal population and cut-off level appropriateness. Based on a cut-off value of 7 U/mL, 90% were negative (mean value 4 U/mL, 2SD = 2.5 U/mL). There is no equivocal (gray) zone for this assay.

2. Comparison studies:
   a. *Method comparison with predicate device:*
      Comparison studies for 62 subjects (40 healthy blood donors and 22 patients with primary biliary cirrhosis (PBC) showed an overall agreement of 90.3%.
   b. *Matrix comparison:*
      Serum is the only recommended matrix.
3. Clinical studies:
   a. *Clinical sensitivity:*
      Clinical sensitivity for the new assay was determined by testing a population of PBC patient serum specimens (n=123). Using a cut-off of 7 U/mL, 111 of the 123 samples (90.2%) were positive for anti-mitochondrial M2 antibodies. The mean value for the PBC samples was 106.7 U/mL. When this values was compared to the mean for the healthy controls, a p-value of 3.93 x 10-51 was obtained (single factor ANOVA). At a level of $p<0.05$ for statistical significance, the results of this population were determined to be statistically different compared to the healthy controls.
   b. *Clinical specificity:*
      A total of 289 samples were tested. Serum samples from 168 consecutive healthy blood donors were used as a normal population. Serum samples from three liver disease groups were tested to further determine clinical specificity of the test. The groups included autoimmune hepatitis type I (n=27) and type II (n=4), hepatitis B virus positive (n=44), and hepatitis C virus positive (n=46). Anti-mitochondrial M2 antibodies are specific for primary biliary cirrhosis and should not be found in normal sera or other liver disease patient sera. Two hundred eighty three samples were found negative in these populations demonstrating a specificity of 98% for the 7 U/mL cut-off value.
   c. *Other clinical supportive data (when a and b are not applicable):*

4. Clinical cut-off:
   See assay cut-off.
5. Expected values/Reference range:
   The expected value in the normal population is negative. According to published literature, the incidence in the PBC group is 90-95%.

M. Conclusion:

The MESACUP-2 Test Mitochondria M2 is substantially equivalent to other devices regulated under 21 CFR §866.5090, product code DMB, Class II